



TITLE:

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CITATION:

Yoshida, Takeshi ...[et al]. Hypergonadotropic hypogonadism and hypersegmented neutrophils in a patient with ataxia-telangiectasia-like disorder: Potential diagnostic clues?. American journal of medical genetics. Part A 2014, 164(7): 1830-1834

ISSUE DATE:

2014-07

URL:

<http://hdl.handle.net/2433/188907>

RIGHT:

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Hypergonadotropic Hypogonadism and Hypersegmented Neutrophils in a Patient With Ataxia-Telangiectasia-like Disorder: Potential Diagnostic Clues?

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Manuscript Received: 9 March 2013; Manuscript Accepted: 27 February 2014

Ataxia-telangiectasia-like disorder (ATLD) is a rare autosomal recessive disorder, and has symptoms similar to ataxia-telangiectasia (AT). ATLD is caused by mutations in the *MRE11* gene, involved in DNA double-strand break repair (DSBR). In contrast to AT, ATLD patients lack key clinical features, such as telangiectasia or immunodeficiency, and are therefore difficult to be diagnosed. We report a female ATLD patient presenting with hypergonadotropic hypogonadism and hypersegmented neutrophils, previously undescribed features in this disorder, and potential diagnostic clues to differentiate ATLD from other conditions. The patient showed slowly progressive cerebellar ataxia from 2 years of age, and MRI revealed atrophy of the cerebellum, oculomotor apraxia, mild cognitive impairment, writing dystonia, hypergonadotropic hypogonadism with primary amenorrhea, and hypersegmented neutrophils. Western blot assay demonstrated total loss of MRE11 and reduction of ATM-dependent phosphorylation; thus, we diagnosed ATLD. Genetically, a novel missense mutation (c.140C>T) was detected in the *MRE11* gene, but no other mutation was found in the patient. Our presenting patient suggests that impaired DSBR may be associated with hypergonadotropic hypogonadism and neutrophil hypersegmentation. In conclusion, when assessing patients with ataxia of unknown cause, ATLD should be considered, and the gonadal state and peripheral blood smear samples evaluated. © 2014 Wiley Periodicals, Inc.

Key words: ataxia-telangiectasia-like disorder; ATLD; *MRE11*; hypergonadotropic hypogonadism; hypersegmented neutrophil

INTRODUCTION

Ataxia-telangiectasia-like disorder (ATLD) is a very rare disease with autosomal recessive inheritance, first reported as a milder form of ataxia-telangiectasia (AT) [Hernandez et al., 1993]. Advances in genetics have revealed that ATLD is caused by mutations in the

How to Cite this Article:

Yoshida T, Awaya T, Shibata M, Kato T, Numabe H, Kobayashi J, Komatsu K, Heike T. 2014. Hypergonadotropic hypogonadism and hypersegmented neutrophils in a patient with ataxia-telangiectasia-like disorder: Potential diagnostic clues?

Am J Med Genet Part A 164A:1830–1834.

MRE11 gene, which is involved in DNA double-strand break repair (DSBR) together with *ATM*, the causative gene in AT [Savitsky et al., 1995; Stewart et al., 1999]. Patients with ATLD show progressive cerebellar ataxia, abnormal eye movement, and increased cellular radiosensitivity, all of which are also observed in AT. However, in contrast to AT, ATLD patients exhibit no extraneurological features, such as telangiectasia, reduced immunoglobulin levels, or raised alpha fetoprotein. The lack of such key clinical features makes it difficult to distinguish this condition from other cerebellar ataxias; hence correct diagnosis is delayed in the majority of cases.

Here, we report an ATLD patient presenting with previously undescribed features; hypergonadotropic hypogonadism and hypersegmented neutrophils. Both of these symptoms have potential as diagnostic clues in differentiating ATLD from other cerebellar ataxias of unknown causes.

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Article first published online in Wiley Online Library (wileyonlinelibrary.com): 14 April 2014

DOI 10.1002/ajmg.a.36546

CLINICAL REPORT

The patient was born to non-consanguineous, healthy, Japanese parents after an uneventful gestation and delivery, and had no history of perinatal or postnatal infections. Her elder brother was healthy and there was no family history of neurological disease or malignancy.

The patient's development was normal during infancy; she was able to sit without support at age 8 months and walked alone at 14 months. Mild unstable gait was noticed at 2 years of age, but cranial computed tomography demonstrated no apparent abnormality. She was then diagnosed with ataxic cerebral palsy and observed periodically. Thereafter, her ataxic symptoms progressed slowly and cranial magnetic resonance imaging (MRI) at 9 years of age revealed mild cerebellar atrophy. As progression of cerebellar atrophy was noted on MRI at 12 years, she was referred to our hospital for further investigation.

The patient weighed 31 kg (−2.5 standard deviation [SD] scores), measured 150.4 cm (−1.2 SD) in height, and had a head circumference of 53 cm (−1.0 SD) at the first visit to our hospital. She had neither dysmorphic features nor skin lesions, including telangiectasia. Neurological examination demonstrated cerebellar ataxia: slurred speech, dysidiadokokinesis, and unsteady gait with a tendency to fall. Finger-to-nose test revealed dysmetria and intention tremor on both sides. She also showed oculomotor apraxia. Her IQ score was assessed with the Wechsler Intelligence Scale for Children III: full scale, 60; verbal, 76; performance, 50. Cranial MRI revealed remarkable cerebellar atrophy (Fig. 1). Serum alpha fetoprotein (AFP) and immunoglobulin (Ig) levels were within the reference range: AFP, 2.0 ng/mL; Ig A, 181.5 mg/dL; Ig G, 983.0 mg/dL; and Ig M, 152.8 mg/dL. Peripheral blood smears showed neutrophils with hypersegmented and dysmorphic nuclei, accounting for 1–3% of total white blood cells (Fig. 2). Her bone marrow smear revealed hypocellular marrow without any signs of malignancy (nucleated cell count, 11,500/μL; megakaryocytes, 3/μL; M/E ratio, 2.5). Abnormal nuclear segmentation was only observed in mature neutrophils. Fundoscopy, nerve conduction study, electromyography, and auditory brainstem response were all normal.

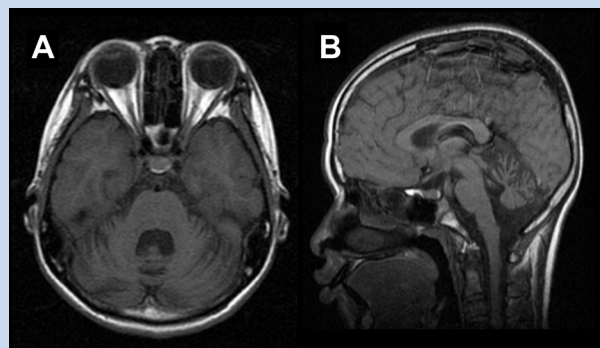


FIG. 1. Cranial MRI at age 15 years showing remarkable cerebellar atrophy: (A) axial T1 weighted image; (B) sagittal T1 weighted image.

Endocrinologically, the patient had almost no secondary sexual characteristics at 12 years of age. According to Tanner's stages, her breasts were stage 2 and her pubic hair stage 1. Laboratory tests showed hypergonadotropic hypogonadism: follicle-stimulating hormone, 114.9 IU/L (normal range for females at 11–14 years, <0.1–12.0); luteinizing hormone, 36.4 IU/L (normal <0.1–13.4); estradiol, 8.5 pg/ml (normal <20–87) [Soldin et al., 2005]. Her ovaries were undetectable by abdominal MRI.

Subsequently, her ataxic symptoms progressed further and at 14 years of age she required a wheelchair. She also developed difficulty in writing due to dystonic involuntary movements of the right hand. She is presently 21-years-old and has been receiving hormone replacement therapy for primary amenorrhea. There has been no evidence of malignancies to date.

GENETIC ANALYSIS

Direct sequencing of all exons of the *MRE11* gene revealed a novel missense mutation (c.140C>T, p.Ala47Val) in exon 3 in the patient and her father (Fig. 3A). No other mutations were found in either the patient or her parents. However, sequencing analysis of mRNA of *MRE11* revealed a single peak for T, indicating that *MRE11* mRNA was expressed only from the mutant allele T in the patient, as opposed to the double peaks for alleles C and T observed in the father's sample (Fig. 3B). This mutation was not found in any public variant database such as dbSNP (<https://www.ncbi.nlm.nih.gov/SNP/>) and JSNP (<http://snp.ims.u-tokyo.ac.jp/>). We also performed comparative genomic hybridization (CGH) to examine small deletions or duplication. The CGH + SNP 180 K array (Agilent Technologies, Santa Clara, CA) data indicated that there was no significant signal change detected within and proximal to the *MRE11* gene.

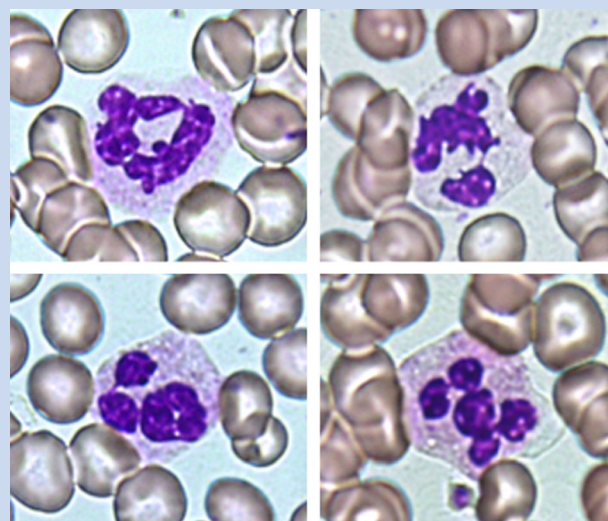


FIG. 2. Peripheral blood smears showing neutrophils with hypersegmented and dysmorphic nuclei.

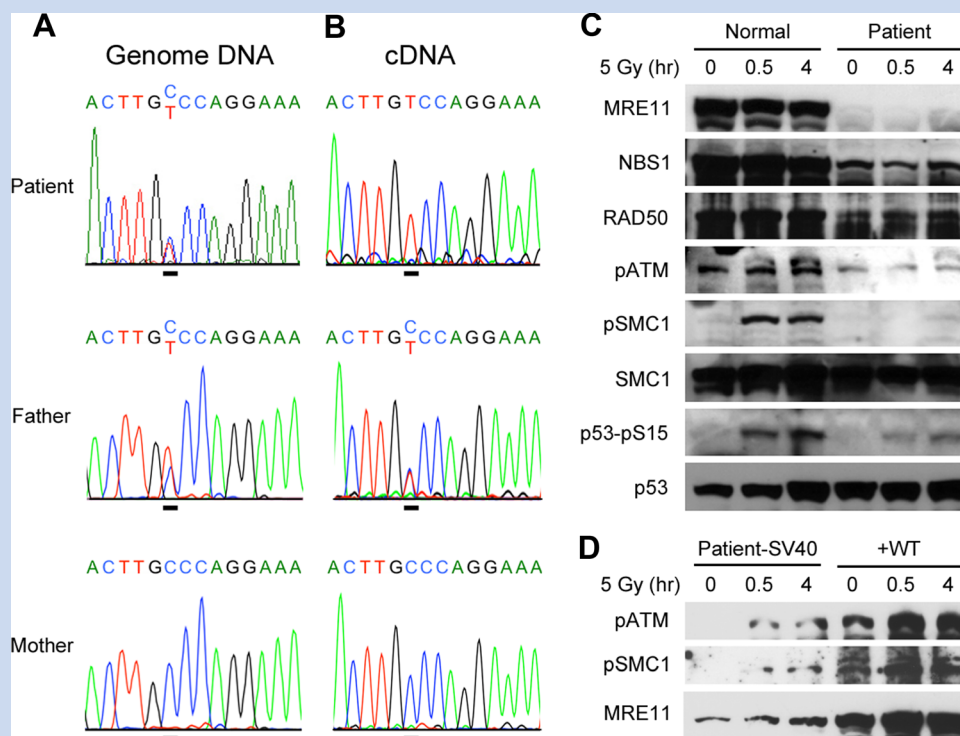


FIG. 3. Genetic analysis of the patient and her parents. **A:** A novel missense mutation [c.140C>T] in exon 3 of the *MRE11* gene was detected in the patient and the father. **B:** The mRNA [cDNA] analysis showing the presence of only the mutated allele-T at c.140, reflecting the absence of mRNA from the other allele in the patient, but the presence of both alleles in the father. **C:** Western blot assay of skin fibroblasts from the patient and a healthy control donor with or without irradiation (5 Gy gamma ray) demonstrating complete absence of MRE11 protein in the patient. There were reduced levels of NBS1 and RAD50. Phosphorylation of ATM [pATM], SMC1 [pSMC1], and p53 [p53-pS15] were remarkably low in the patient, and did not increase sufficiently after irradiation. **D:** ATM phosphorylation pathway was rescued by introducing wild-type *MRE11* gene. In the SV40-transformed patient fibroblasts, MRE11 protein expression was remarkably reduced and the deficit of ATM phosphorylation after irradiation was also prominent. The introduction of wild-type *MRE11* gene restored the MRE11 protein levels and the phosphorylation of both ATM [pATM] and SMC [pSMC], indicating that the other members of MRN complex functioned normally.

Western Blot ASSAY

Skin fibroblasts were obtained from the patient and from a healthy donor control. The cells were irradiated with 5 Gy of gamma rays, and then whole cell extracts were analyzed by western blot assay to determine MRE11, RAD50, and NBS1 levels. In addition, we assayed for irradiation-stimulating phosphorylation of ATM (pATM), SMC1 (pSMC1), and p53 at serine 15 (p53-pS15), all of which are involved in the signaling pathway initiated by activation of ATM, also by western blotting. Expression of MRE11 protein was almost completely absent from fibroblasts derived from the patient, both before and after irradiation (Fig. 3C). The patient showed significantly reduced levels of NBS1 and RAD50 proteins, which, together with MRE11, form the MRE11/RAD50/NBS1 (MRN) complex [Czornak et al., 2008]. Bands representing pATM, pSMC1, and p53-pS15 were also markedly reduced in the patient compared to the control. These results demonstrate that absence of the MRE11 protein unstabilized the MRN complex, leading to reduced activation of ATM and proteins downstream of ATM, such as SMC1 and p53.

We also examined whether the restoration of ATM phosphorylation was rescued by introducing the wild-type *MRE11* gene into the cells. In this experiment, we used SV40-transformed fibroblasts. SV40 transformation increases ATM and MRN complex in cultured fibroblasts; therefore, MRE11, which was not detected in the non-transformed patient's fibroblasts (Fig. 3C), was weakly detected by western blotting assay. However, phosphorylation of ATM and SMC after irradiation was markedly decreased, indicating that MRE11 protein of the patient was non-functioning. Both ATM and SMC phosphorylation was restored by introduction of wild-type *MRE11*, indicating that other members of MRN complex functioned normally (Fig. 3D).

DISCUSSION

Here, we report a female patient with the following clinical features: progressive cerebellar ataxia, oculomotor apraxia, writing dystonia, hypergonadotropic hypogonadism, and hypersegmented neutrophils.

Genetic analysis identified a missense mutation, c.140C>T (p. Ala47Val), in *MRE11* both the patient and her father. The

mutation of the 47th alanine residue is highly likely to be pathogenic as it is located within the important nuclease domain and is highly conserved from plants to humans [Park et al., 2011]. We could not detect any other mutations as far as we investigated; however, the patient's mRNA was expressed only from the mutated T-allele and, in contrast, the father's mRNA was expressed from both of the mutated T-allele and the other wild-type C-allele. As ATLD is an autosomal recessive disorder, the most plausible explanation is that the patient is a compound heterozygote for *MRE11* comprising the paternally derived mutated allele-T and an unidentified mutation on the other normal-looking C-allele bearing chromosome that might be derived from her mother. Such an unidentified alteration may include a promoter region mutation, an epigenetic alteration, or an intronic mutation. This hidden mutated allele of the patient would not express mRNA, thus, mRNA is expressed as if it comes only from the mutated T-allele. We analyzed MRE11 protein levels and mRNA expression in the patient and her parents, but the results were inconclusive. A similar result was described in a previous report [Stewart et al., 1999]. We speculate mRNA expression is likely increased through a feedback mechanism, and the presence of one normal allele is sufficient to maintain normal protein levels, as observed in the father's sample. Western blot assay revealed an almost complete absence of MRE11 and reduced expression of associated proteins in the patient, even after irradiation. Furthermore, the deficit in ATM phosphorylation pathway was rescued by introducing wild-type *MRE11* gene into the patient's fibroblasts, indicating that the lack of MRE11 was pathogenic in this case. Based on these data, we diagnosed ATLD, which is caused by MRE11 deficiency.

The association between cerebellar ataxia and hypogonadism has been described as Gordon-Holmes cerebellar ataxia for more than 100 years [Holmes, 1908]. The classification Gordon-Holmes type cerebellar ataxia is used to describe individuals presenting with either hypogonadotropic or hypergonadotropic hypogonadism; however, these two conditions are completely different [Amor et al., 2001; Seminara et al., 2002]. Hypogonadotropic hypogonadism is much more frequent and is secondary to reduced pituitary production of gonadotropins. By contrast, hypergonadotropic hypogonadism means primary dysfunction of the ovary or testis.

Due to the rarity of ATLD, the gonadal status of patients has not been well described. In the case of AT, some female patients have been reported to show primary ovarian failure [Zadik et al., 1978]. In addition, *Atm*-deficient mice, models of AT, demonstrate primary gonadal failure, including significantly atrophied gonads, decreased levels of testosterone in males or decreased estradiol in females, and elevated levels of follicle-stimulating hormone [Rasheed et al., 2006]. These data suggest that the hypogonadism observed in AT patients is essentially a result of primary gonadal failure; although accompanying secondary gonadal failure due to pituitary dysfunction can mask primary gonadal failure. According to a study of *Atm*-deficient mice, *Atm* is necessary for appropriate meiotic DNA recombination, and the authors speculate that *Atm* is involved in the repair of DNA breaks during meiotic recombination [Barlow et al., 1998]. As ATM and MRE11 proteins are known to interact, and both play critical roles in the DSB repair pathway, ATLD

patients may present with hypergonadotropic hypogonadism, similar to AT patients.

Hypersegmented neutrophils are also known to occur in several conditions that affect DNA replication, such as vitamin B12 deficiency, folate deficiency, or other DNA damaging conditions. Similarly, hypersegmentation of neutrophils in our patient could be the result of impaired DSB repair, though the underlying mechanism remains to be established.

Unlike AT, extraneurological symptoms are not usually described in patients diagnosed with ATLD, and this can lead to delays in reaching a correct diagnosis. Uchisaka et al. [2009] recently reported that two brothers with ATLD developed lung adenocarcinoma during childhood, indicating the importance of early diagnosis and careful observation for malignancies in patients with ATLD, as well as those with AT.

In this study, we describe hypergonadotropic hypogonadism and hypersegmented neutrophils, which are features previously unreported in ATLD. It suggests that impaired DSB repair may be associated with hypergonadotropic hypogonadism and neutrophil hypersegmentation. Our conclusion is that clinicians should consider the possibility of ATLD and other DNA repair disorders, when assessing patients presenting with ataxia of unknown cause, and evaluate the gonadal state and peripheral blood smear samples of patients.

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